

CLAIMS

WE CLAIM:

1. A method for producing and evaluating a bioactive molecule comprising the steps of:
 - a) providing a nucleic acid sequence comprising a bioactive molecule;
 - b) expressing the bioactive molecule encoded by the nucleic acid sequence obtained in step (a), wherein the expressed bioactive molecule has a detectable phenotype;
 - c) contacting the bioactive molecule obtained in step (b) with a compound; and
 - d) detecting the phenotype of the bioactive molecule in the presence or absence of the compound contacted in step (c).
2. The method of claim 1, wherein the bioactive molecule is selected from the group consisting of: a viral molecule, a bacterial molecule, a fungal molecule, a protozoal molecule, a human molecule and an animal molecule.
3. The method of claim 1, wherein the bioactive molecule is a protein further comprising a retrovirus protein, a herpesvirus protein, a hantavirus protein, a hepatitis virus protein, an influenza protein, a myxovirus protein, a picornavirus protein, an adenovirus protein, a poxvirus protein, a flavivirus protein or a coronavirus protein.
4. The method of claim 1, wherein the bioactive molecule is a protein further comprising a streptococcus protein, a staphylococcus protein, an enterococcus protein, a neisseria protein, a salmonella protein, a mycobacteria protein, a bacillus protein, a mycoplasma protein, a chlamydia protein, a francisella protein, a pasteurilla protein, a brucella protein,

a pseudomonas protein, a listeria protein, a clostridium protein, a yersinia protein, a vibrio protein, a shigella protein, or an enterobacteriaceae protein.

5. The method of claim 1, wherein the bioactive molecule is a protein further comprising a plasmodium protein, a trypanosome protein, or a cryptosporidium protein.
6. The method of claim 1, wherein the bioactive molecule is a protein further comprising a candida protein, a cryptococcus protein, a malassezia protein, a histoplasma protein, a coccidioides protein, a hyphomyces protein, a blastomyces protein, an aspergillus protein, a penicillium protein, a pseudallescheria protein, a fusarium protein, a paecilomyces protein, a mucor/rhizopus protein, a pneumocystis protein, a rhinosporidium protein, a sporothrix protein, a trichophyton protein, a microsporum protein, an epidermophyton protein, a basidiobolus protein, a conidiobolus protein, a rhizopus protein, a Cunninghamella protein, a paracoccidioides protein, a pseudallescheria protein, or a rhinosporidium protein.
7. The method of claim 1, wherein the nucleic acid sequence encoding the biomolecule further comprises deoxyribonucleic acid or ribonucleic acid.
8. The method of claim 1 or claim 7, wherein the nucleic acid sequence encoding a bioactive molecule further comprises transfer RNA or polyA⁺ RNA.
9. The method of claim 1, wherein the bioactive molecule further comprises a protein, a glycoprotein, a polysaccharide, a mucopolysaccharide, a lipopolysaccharide, a lipoprotein, a carbohydrate, or a nucleic acid.

10. The method of claim 1, wherein the bioactive molecule encoded by the nucleic acid is expressed in a cell-free eukaryotic cell lysate translation system.
11. The method of claim 1, wherein the bioactive molecule encoded by the nucleic acid is expressed in a cell-free prokaryotic cell lysate translation system.
12. The method of claim 10, wherein the bioactive molecule encoded by the amplified nucleic acid sequence is expressed in a cell-free reticulocyte lysate translation system.
13. The method of claim 12, wherein the bioactive molecule encoded by the amplified nucleic acid sequence is expressed in a cell-free reticulocyte lysate coupled transcription/translation system.
14. The method of claim 13, wherein the bioactive molecule encoded by the nucleic acid sequence and expressed in a cell-free reticulocyte lysate coupled transcription/translation system is a nucleic acid selected from the group consisting of: deoxyribonucleic acid, ribonucleic acid, polyA+ RNA, tRNA, and rRNA.
15. The method of claim 1, wherein the nucleic acid sequence that encodes the bioactive molecule further comprises a second nucleic acid sequence operably linked to said bioactive molecule.
16. The method of claim 15, wherein the second nucleic acid sequence comprises a regulatory element.

17. The method of claim 15, wherein the second nucleic acid sequence comprises a purification motif.
18. The method of claim 15, wherein the second nucleic acid sequence encodes a gene product or fragment thereof comprising a purification motif.
19. The method of claim 1, wherein the bioactive molecule is contacted with a compound selected from the group consisting of: an anti-viral compound, an anti-bacterial compound, an anti-fungal compound, an anti-cancer compound, an immunosuppressive compound, a hormone, a cytokine, a lymphokine, a chemokine, an enzyme, a polypeptide, a polynucleotide, and a nucleoside analogue.
20. The method of claim 1, wherein detecting the phenotype of the bioactive molecule further comprises assaying the enzymatic activity of the bioactive molecule.
21. The method of claim 20, wherein assaying the enzymatic activity of the bioactive molecule further comprises assaying the bioactive molecule for a resistance phenotype to the compound.
22. The method of claim 1, wherein detecting the phenotype of the bioactive molecule further comprises assaying the affinity of the bioactive molecule for the compound.
23. The method of claim 22, wherein assaying the affinity of the bioactive molecule for the compound further comprises assaying the bioactive molecule for a resistance phenotype to the compound.

24. The method of claim 1, wherein detecting the phenotype of the bioactive molecule further comprises assaying the structure of the bioactive molecule.
25. The method of claim 24, wherein assaying the structure of the bioactive molecule comprises predicting a resistance phenotype to the compound.
26. The method of claim 1, wherein the method is preceded by the step of: amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid sequence comprises a bioactive molecule.
27. The method of claim 1, wherein the nucleic acid encoding a bioactive molecule is amplified by a reaction selected from the group consisting of: a polymerase chain reaction, a ligase chain reaction, a transcription mediated amplification reaction, a nucleic acid sequence based amplification reaction, and a strand displacement amplification reaction.
28. The method of claim 1, wherein amplifying the nucleic acid encoding the biomolecule comprises a polymerase chain reaction further comprising one or more nested primer sets.
29. The method of claim 1, wherein amplifying the nucleic acid encoding the biomolecule comprises oligonucleotide primers comprising the sequences of SEQ ID NO.:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4.
30. The method of claim 1 or claim 26, wherein the method is preceded by the step of: extracting one or more specimens from a patient afflicted with a disease state, wherein the specimens comprise a bioactive molecule associated with a disease state.

31. A method for producing and evaluating a bioactive molecule comprising the steps of:
- a) isolating at least one organism or tissue, wherein the organism or tissue comprises a bioactive molecule associated with a disease state;
 - b) amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid sequence comprises the bioactive molecule and is obtained from the organism or tissue isolated in step (a);
 - c) expressing the bioactive molecule encoded by the nucleic acid sequence obtained in step (b), wherein the expressed bioactive molecule has a detectable phenotype further comprising resistance to a first compound;
 - d) contacting the bioactive molecule obtained in step (c) with a second compound; and
 - e) detecting the phenotype of the bioactive molecule in the presence or absence of the second compound contacted in step (d).
32. The method of claim 1, wherein the method is preceded by the step of: amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid sequence comprises a bioactive molecule.

33. The method of claim 1 or claim 26, wherein the method is preceded by the step of :
extracting one or more specimens from a patient afflicted with a disease state, wherein
the specimens comprise a bioactive molecule associated with the disease state.
34. A method for producing and evaluating a bioactive molecule comprising the steps of:
- a) isolating at least one organism or tissue, wherein the organism or tissue comprises a
bioactive molecule associated with a disease state;
 - b) amplifying a nucleic acid sequence in a cell-free system, wherein the nucleic acid
sequence comprises the bioactive molecule and is obtained from the organism or
tissue isolated in step (a);
 - c) expressing the bioactive molecule encoded by the nucleic acid sequence obtained in
step (b), wherein the expressed bioactive molecule has a detectable phenotype further
comprising resistance to a first compound;
 - d) contacting the bioactive molecule obtained in step (c) with a second compound; and
 - e) detecting the phenotype of the bioactive molecule in the presence or absence of the
second compound contacted in step (d).

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